PROTECTIVE EFFECTS OF FLUORIDE AND AMORPHOUS CALCIUM PHOSPHATE AGAINST ACID EROSION

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MASTER'S THESIS

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ABSTRACT

PROTECTIVE EFFECTS OF FLUORIDE AND AMORPHOUS CALCIUM PHOSPHATE

AGAINST ACID EROSION

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Background: Erosion is an increasing dental health challenge due to the irreversible effects of enamel loss. Fluoride (F) is a proven therapy for its remineralization effects. Recent studies have suggested that CPP-ACP (casein phosphopeptide–amorphous calcium phosphate) may provide similar protection against acid erosion.

Objective: The aim of this pilot study was to compare the enamel-protective effects of fluoride and CPP-ACP against erosive challenges that simulate prolonged exposure to acidic conditions.

Methods: Ten de-identified extracted human teeth were sectioned and sanded into 100-micron sections, then embedded along with TEM grids as geometric markers in epoxy to expose a flat enamel edge. A total of 75 specimens were subjected to a daily pH-cycling challenge, alternating between artificial saliva (pH 7.0; 23.5 hours) and citric acid (pH 3.9; 30 minutes). Four concentrations of protective agents (900 ppm F, 5000 ppm F, CPP-ACP, and CPP-ACP + 900 ppm F; control = no treatment) were applied immediately following the acidic

challenge at three time intervals (weekly, 3 times per week, and daily applications; n = 5 specimens per treatment group/frequency). Microradiographs were taken before experimentation (baseline) and following 1 week and 2 weeks of acid exposure/treatment. Radiographs were digitized and viewed under a stereomicroscope to quantify enamel surface erosion.

Results: The results demonstrated that daily applications of various F and ACP therapies were more protective than 3 times per week and weekly applications (two-way ANOVA; $p < 0.05$). Fluoride 5000 ppm applied daily was most effective against acid erosion. The combination of fluoride and CPP-ACP did not appear to improve the protective effects of either agent alone.

Conclusions: Based on the results of this pilot study, it may be beneficial to prescribe fluoride 5000 ppm for daily application to reduce the risk of dental erosion.

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LIST OF ABBREVIATIONS

CAP	carbonated hydroxyapatite
Ca^{2+}	calcium
$CO_3^{=}$	carbonate
CPP-ACP	casein phosphopeptide-amorphous calcium phosphate
HAP	hydroxyapatite
F	fluoride
NaF	sodium fluoride
PO_4^{3-}	phosphate
PPM or ppm	parts per million

CHAPTER 1: REVIEW OF LITERATURE

Erosion

Dental enamel is the hardest substance in the human body. Yet, human behavior can lead to structural damage in the enamel via chemical exposure, mechanical wear and bacterial attack. Review of current literature demonstrates a need to clarify the terminologies that define such damages. Tooth wear can come in the form of erosion, abrasion and attrition (Rose, Haverman & Davis 2006; Abrahamsen 2005; Bartlett 2005). Attrition results from tooth to tooth contact during function, while abrasion is caused by mechanical wear via a foreign medium, such as toothpaste or aggressive tooth brushing habits (Lussi 2006; Abrahamsen 2005; Rose & colleagues 2006). Dental erosion is defined as an irreversible loss of surface dental tissue from acid exposure, independent of bacterial involvement (Lussi 2006; Balooch & colleagues 1995).

On the other hand, the metabolic acids produced by intraoral bacterial plaque cause dental caries. While erosion destruction takes place on the tooth surface, caries demineralization can occur on the surface, within the subsurface region, or in both areas simultaneously (Lussi 2006; Magalhaes & colleagues 2009).

Erosion is likely a multifactorial phenomenon that is influenced by an individual's consumption habits, as well as biological and chemical factors (Lussi 2006). Acid exposure, intrinsic or extrinsic, is thought to be the main etiology of erosive dissolution of calcium (Ca^{2+}) and phosphate (PO_4^{3-}) from tooth enamel (Rose & colleagues 2006; Rees, Loyn & McAndrew 2005; Bartlett 2005). Initially, a surface softened layer results from the loss of calcium and phosphate. The softened layer of enamel is more susceptible to caries

formation, erosion, and mechanical abrasion from toothbrush or toothpaste (Lussi 2006; Abrahamsen 2005; Rose & colleagues 2006).

Intrinsic acidic sources include gastric acids (hydrochloric acid) that enter the oral cavity, as seen in gastric esophageal reflux disease (GERD) and eating disorders such as bulimia (Abrahamsen 2005; Rose & colleagues 2006; Lussi 2006). Both of these conditions can cause generalized dentinal sensitivity and severe erosion, particularly on the lingual surfaces of anterior teeth and occlusals of posterior teeth (Abrahamsen 2005).

Acidic foods and beverages are common sources of extrinsic acid exposure. Energy and sports drinks are prime examples because citric acid, along with other acidic additives, is a common ingredient (Coombes 2005; Kitchens & Owens 2007; www.energyfiend cited 2010 Dec 23). In recent years, energy drinks have gained much popularity among young adults and military service members. According to the National Association of Convenience Sales (NACS), energy drink sales have increased 400% from 2002 to 2007 to $6.6 billion dollars and are expected to exceed $9 billion in 2011 (www.nacsonline.com, 2010 Dec 2). The demand is evident in the wide selection of beverages (e.g., Monster, Red Bull, Full Throttle, and others) available in military commissaries, Army and Air Force Exchange Service (AAFES) and Navy Exchange (NEX) package stores (www.military.com 2010 Dec 23). AAFES reported sales in 2008 of over 2.4 million containers of energy drinks, with Monster being the number one seller (www.stripes.com 2010 Dec 23, www.military.com 2011 Dec 23).

The potential destruction caused by acidic drinks can be challenging to treat due to the frequency, duration of exposure, and habitual patterns of consumption (West, Hughes & Addy 2000; Lussi & Jaeggi 2008). Many patients with generalized erosion admit to constant

sipping and swishing of the beverage or holding the carbonated drinks in their mouth until the bubbles subside before swallowing (Bartlett 2005; Lussi 2006). These habits increase the contact time of the acidic beverages on the teeth and maintain an oral environment below the critical pH of 5.5. At this low pH, the protective buffering factors in saliva, calcium, and phosphate are ineffective, creating a perpetual acidic environment that favors enamel demineralization. Initially, the loss of mineral from the enamel may cause surface dulling, lack of luster and softening (Lussi 2006). Eventually, if the process continues, it can lead to dentinal sensitivity, generalized erosion, rampant caries, and loss of occlusal vertical dimension and restorative space (Abrahamsen 2005, Rose & colleagues 2006, Bartlett 2005). Treatment options are complex and may require full mouth rehabilitation due to the generalized extensive destructive patterns. Therefore, early recognition, prevention, and remineralization of early enamel lesions are keys to avoid extensive dental restorations. The calcium and phosphate lost during demineralization must be replenished by some form of remineralization therapy to prevent surface erosion and progression of lesions into the dentin.

Enamel Structure

Enamel is composed of carbonated hydroxyapatite (CAP), water and small amounts of proteins and lipids. The carbonated hydroxyapatite is more acid soluble compared to regular hydroxyapatite (HAP) due to the replacement of calcium ions by metal ions, such as sodium, magnesium, and potassium. These carbonate-rich regions of the enamel contain areas where the substitution of carbonate ($CO_3^{=}$) for some of the phosphate (PO_4^{3-}) disturbs the crystal lattice structure. The irregular crystalline structure is a major contributor to

potential acid solubility. Dentin is more susceptible to acidic attack because it has more carbonate content and smaller HAP crystals than enamel, creating more exposed surface area (Featherstone 2000; Lussi & Jaeggi 2008).

Dissolution and chelation are two methods by which calcium and phosphate are leached from the tooth surface during acid exposure. The hydrogen ions that dissociate from an acid can attack the crystalline surface of enamel and combine with the carbonate and phosphate ions, causing these ions to dissolve from the tooth surface (Lussi & Jaeggi 2008). In addition, when teeth are exposed to weak acids, such as citric acid, the acid further promotes demineralization by acting as a chelator with its acid anions (COO^-) and binding the calcium (Ca^{2+}) in the saliva (Lussi 2006; Sauro 2008; von Fraunhofer & Rogers 2004). This reduces the saliva's concentration of calcium and buffering capacity. When the softened apatite crystals collapse, a surface enamel layer is removed, leading to erosion (Wang & colleagues 2010).

Measurement of Enamel Loss

The ability to identify early erosion and quantify the mineral density in enamel is valuable in understanding the demineralization process and evaluating the efficacy of remineralization treatment. Various techniques are useful to quantitatively and qualitatively assess the softened surface and enamel loss due to acidic challenges. There are several suitable methods (Attin 2006):

- Scanning electron microscopy (SEM) qualitatively detects surface alterations.

- Surface hardness measurement assesses initial weakening on the surface by using either Knoop or Vickers varying indentation depths on polished tooth surfaces to calculate the amount of enamel surface loss.
- Surface profilometry uses a laser beam or contact stylus to scan and map surface loss and roughness.
- Atomic force microscopy uses high resolution imaging to measure size differences in atoms on the substrate surface.
- Nanoindentation uses an indenter diamond to assess mechanical properties on softened surfaces.
- Microradiography provides an analysis of the dissolved minerals on the enamel and dentin surface.

Microradiography has been traditionally used to measure mineral changes in subsurface lesions typical in early dental caries. It reveals areas of mineral density based on the attenuation of X-ray energy transmitted by mineral. Demineralized areas are seen as radiolucent zones, while remineralized areas appear as radiopaque zones. In addition, microradiography has been adopted to assess erosive mineral loss. The primary advantage of this method is that it allows for simultaneous analysis of both erosive surface loss and demineralization (Attins 2006). Several studies have utilized microradiography as the method of choice to visualize and analyze mineral density. Wefel and Harless (1984) compared artificially demineralized white spot lesions using contact microradiography and polarized light microscopy to assess the ability of three artificial acid/caries systems to replicate natural white spot lesions on extracted molars. Chow and Takagi (1995) were able

to quantify the mineral content in root caries lesions before and after remineralization treatment with concentrated calcium and phosphate solutions. Iijima and Koulourides (1988) used microradiography to visualize the development of radiopaque zones of remineralization in enamel lesions following serial remineralization treatments.

Recently, Schmuck and Carey (2010) demonstrated the accuracy of an improved microradiographic technique, which allows direct measurement of surface erosion depth and quantifies the change in mineral density in the enamel following remineralization treatment. While the previously used film, Kodak Professional Film SO343 (Eastman Kodak, Co., Rochester, NY) has been discontinued in the market, they successfully tested the accuracy of a new (VRP-M) holographic film that provides greater than 3000 lines per millimeter resolution power.

Fluoride

Fluoride's protective effects, as seen in dentifrice and water fluoridation, have been widely successful for caries control and remineralization (Rose & colleagues 2006; Featherstone 2000). The Centers for Disease Control (1999) has credited fluoridation of drinking water among the 10 greatest achievements of modern public health initiative for its role in reducing the prevalence and incidence of dental caries. Fluoride is effective via three modes of actions: (1) it inhibits demineralization (mineral loss) during episodes of acid exposure; (2) it enhances remineralization (calcium and phosphate reuptake) and formation of a new crystalline structure, fluorapatite, that excludes the carbonate group, creating a more acid resistant veneer layer on the enamel surface; and (3) in acidic conditions, fluoride can cross bacterial cell membranes and inhibit the bacteria's ability to metabolize

carbohydrates (ten Cate 1991; Featherstone 2000).

High-concentration topical fluoride agents, including dentifrices and gels, which contain 5000 parts per million (ppm) of fluoride, are available by prescription for daily at-home use. These products possess up to five times the fluoride levels of commercial over-the-counter (OTC) dentifrices, which typically contain 1100-1500 ppm fluoride.
Many studies have demonstrated that higher concentrations of fluoride improve remineralization potential and may protect against erosive demineralization of enamel (Mukai, Lagerweij & ten Cate 2001; Baysan and colleagues 2001; Bizhang and colleagues 2009; Garcia and colleagues 2010). In an *in vitro* study, Ganss and colleagues (2001) reported that increased frequency of fluoride rinse application (SnF_2, 0.025% F^-; three 5-minute applications daily) coupled with higher concentration fluoride gel (NaF, 1.25% F^-; one 5-minute application daily) reduced mineral loss in enamel and dentin specimens that were exposed to citric acid cycling; this fluoride regimen appeared to be more protective in dentin than enamel. Ten Cate and colleagues (2008) revealed similar fluoride capacity when they examined the efficacy of various fluoride concentrations implementing a pH cycling apparatus. Treatment with 5000 ppm showed 31% more subsurface remineralization and 12% less demineralization than a 1500 ppm OTC dentifrice equivalent. In addition, Sauro (2008) was able to visualize demineralized enamel prisms with SEM immediately after acidic drink exposure and demonstrated that immediate fluoride dentifrice application protected the surface enamel prisms, with little to no surface morphological change after the acid challenge.

Other studies, however, have not demonstrated that high concentration fluoride can

combat acid erosion (Karlinsey & colleagues 2011). In a double-blind, cross-over study of 10 subjects, Rios and colleagues (2008) created erosion *in situ* by having participants submerge enamel slabs embedded in oral appliances in cola for a one-minute daily acid challenge before returning the appliances to their mouths for fluoride therapy and continual wear. The participants' daily diets were not taken into consideration or restricted; however, they were advised to avoid fluoridated products other than the ones assigned in the study. Enamel loss was determined by prolifometry. Results indicated that brushing four times a day with 5000 ppm fluoride dentifrice slurry for one week during the acid challenge, although slightly better, did not demonstrate a significantly more protective effect than the 1100 ppm OTC equivalent or the placebo dentifrice with no fluoride. However, the reduced fluoride contact time (30 seconds; four applications daily), as well as the limited number of participants may have contributed to the inability to show a significant difference in fluoride's efficacy. In a similar double-blind cross-over study, Magalhaes and colleagues (2008) found that an 1100 ppm fluoride slurry reduced dentin erosion, but a higher concentration of 5000 ppm did not significantly increase the protective effect. These studies are consistent with Silverstone's (1982) findings, which suggest that frequent low dose fluoride is necessary for remineralization, but that the use of higher concentration fluoride may not be better. In addition, Larsen and Richards (2002) showed that the addition of calcium fluoride (up to 20 ppm) to soft drinks did not prevent development of erosion *in vitro*.

Although fluoride's ability to enhance the remineralization of incipient caries lesions is well-documented, the protective effect against enamel and dentin erosion is less clear

(Silverstone 1982; Pearce 1985; Featherstone 2006; Walsh 2009). There is clearly a need to re-examine the methodology of the investigations and protocols used to apply the high concentration fluoride treatment.

Casein Phosphopeptide Complex-Amorphous Calcium Phosphate

Calcium and phosphate ions are the building blocks necessary for the remineralization of tooth structure. The formation of hydroxyapatite is thought to involve the formation and conversion of highly reactive intermediate calcium-phosphate compounds, called amorphous calcium phosphates (ACPs). ACPs [$Ca_3(PO_4)_2$] contain a high concentration of calcium and phosphate ions that have the potential to replace minerals lost during acid challenges. They are formed by combining salts of calcium and phosphate (calcium sulfate and dipotassium phosphate) to form a non-crystalline, amorphous state. ACPs also have the capacity to hydrolyze *in situ* to form apatite crystals in the tooth (Tung & Eichmiller 1999). This process occurs within 6 minutes in neutral pH, which is 20,000 times faster than the formation of HAP in physiologic conditions (Tung & Eichmiller 2004). This apatite forming ability suggests that ACP is a viable ingredient to fill enamel surface defects, and possibly restore the loss of enamel luster, caused by demineralization.

ACPs are insoluble under neutral or alkaline conditions; however, they are highly soluble in acidic conditions. Hence, continuous acidic challenges will rapidly wash the calcium and potassium away. In commercial products, ACPs are stabilized in a useable form by binding them to casein phosphopeptides (CPP). CPPs are derived from cow's milk; they constitute up to 80% of the protein in cow's milk and up to 65% of the protein in human milk (Kunz 1990; Azarpazhooh & Limeback 2009).

The combination of ACP with CCP forms a complex that binds the ACP to the pellicle and bacteria on the tooth's surface (Reynolds 2009). The CPP-ACP complex offers a supersaturated solution of calcium and phosphate ions around the tooth surface to enhance remineralization of white spot lesions. This localizes the CPP-ACP in close proximity to the tooth, and in acidic conditions, such as acidic beverage exposure, buffers the tooth with the free calcium and phosphate ions. Therefore, a state of super-saturation exists that may inhibit enamel demineralization and enhance remineralization. An example of a commercially available CPP-ACP is MI Paste with Recaldent® (GC America).

Recent studies have shown promising results with various applications of CPP-ACP complex. Reynolds (1997) treated enamel sections with 1% CPP-ACP in sugar-free, chewing gum for 14 days and demonstrated remineralization of subsurface demineralization. Oshiro and colleagues (2007), in a 28-day investigation using bovine teeth, demonstrated that applying CPP-ACP twice a day immediately after a 10-minute immersion in lactic acid inhibited erosion on enamel surfaces. When evaluated with field emission SEM, there was minimal surface morphology alteration on enamel treated with CPP-ACP compared to the control group that received no acid challenge or CPP-ACP treatment. Walker and colleagues (2006) concluded that drinking milk fortified with ACP-CCP remineralized *in situ* subsurface lesions in 10 human participants who wore removable appliances containing demineralized enamel sections. Using microradiography, they found that the increase in mineral content was dose dependent, with milk containing 0.2% CPP-ACP and 0.3% CPP-ACP resulting in an increase in mineral content of 81% and 164%, respectively, relative to the control milk. Panich & Poolthong (2009) investigated the effects of CPP-ACP on microhardness of 40

extracted teeth that were alternately immersed in carbonated cola and artificial saliva for 10 cycles of five seconds each, repeated at 6 and 12 hours. They confirmed that a three-minute application of CPP-ACP after each acid challenge significantly increased tooth surface hardness by 13%, as compared to storage in artificial saliva.

On the other hand, Pulido and colleagues (2008) reported that CPP-ACP failed to inhibit the progression of *in vitro* subsurface demineralized lesions on sectioned enamel specimens. After six days in a pH cycling model and daily applications of different remineralization therapies, 5000 ppm fluoride demonstrated a greater protective effect against demineralization than CPP-ACP. In this study the application of CPP-ACP and artificial saliva resulted in no significant difference in subsurface demineralization lesion size.

Research to date, although somewhat limited, suggests that adding CPP-ACP to sports drinks may be effective in reducing erosive potential. Ramalingam, Messer and Reynolds (2009) measured the enamel surface erosion caused by a sports drink containing 0.063%, 0.09%, 0.125%, and 0.25% concentrations of CPP-ACP. Thirty teeth sections were polished and covered with varnish for the control area, while a 1-mm square test window was left exposed to the erosive sports drink immersion for 30 minutes. The results, measured by both SEM and surface profile scan, revealed that the sports drink containing at least 0.09% CPP-ACP had minimal detectable erosion. With 0.25% CPP-ACP added, the enamel surface was indistinguishable from control enamel when viewed at 4000X in SEM. Moreover, a panel of 20 untrained tasters could not distinguish a significant difference in taste between the original sports drink and the ones containing up to 0.125% CPP-ACP. However, the investigators noted the study did not simulate intraoral conditions because it

did not incorporate consumption actions such as sipping and swallowing, or protective factors such as salivary flow and buffering capacity.

CPP-ACP may be useful, either alone or in combination with prescription topical fluorides, for caries-susceptible patients. However, a protocol for the prevention or treatment of either dental caries or erosion has not been established. Recently, CPP-ACPF, which contains both CPP-ACP plus a 900 ppm fluoride paste, has been marketed (www.gcasia.info cited 2011 May 26). Wang and colleagues (2010) concluded that two commercial brands of CPP-ACP, one containing 900 ppm fluoride and one containing no added fluoride, failed to show remineralization potential regardless of the fluoride content in the product. They applied slurries of the test pastes for three minutes, then created surface softening by immersing 90 extracted teeth sections in orange juice for three minutes under constant agitation. Statistical analysis using surface nanohardness (SNH) revealed that none of the commercial products was protective against the erosive challenge. Badr and colleagues (2010) compared the protective effects of 1.23% acidulated phosphate fluoride gel (APF), 0.1% sodium fluoride varnish (NaF), and 0.2% CPP-ACPF in artificially induced erosion on extracted primary and permanent teeth. Thirty primary molars and 30 permanent premolar tooth sections were subjected to 14 days of pH cycling for 6 times per day (5 minutes cola immersion and 30 minutes in artificial saliva). APF gel and CPP-ACPF paste were applied topically for 4 minutes, while the NaF varnish was left to on enamel surface for 12 hours to simulate clinical applications. They measured the change in Vicker's surface microhardness scores following the acid challenge. Results revealed that, on permanent teeth, CPP-ACPF and APF gel demonstrated significantly better protection

against surface softening than fluoride varnish. Meanwhile, primary teeth treated with APF gel had the least percentage change in Vicker's microhardness, possibly indicating that APF provided the most protection on primary teeth.

In addition, Jayarajan and colleagues (2011) compared the remineralization effects of CPP-ACP against CPP-ACPF (900 ppm NaF). They created white spot lesions by submersing 90 sectioned premolars in a demineralization solution for 5 hours and treated them with 4 minutes topical application of CPP-ACP and CPP-ACPF or stored them artificial saliva as the control. Remineralization was analyzed by scanning electron microscope and laser fluorescence. They noted that white spot lesions are usually not visually detected unless they are at least 200-300 μm into enamel. Their investigation revealed that both CPP-ACP and CPP-ACPF showed remineralization potential on white spot lesions based on mineral deposits seen on surface morphology at 2000x magnification; however, while CPP-ACPF demonstrated slightly more remineralization, it was not a significant amount.

Summary

Dental erosion is a potentially increasing risk among people who persistently sip sports drinks for hydration and energy drinks for stamina. There is a critical need to address the challenges that arise from the habitual consumption of these acidic beverages. Since intraoral conditions are complex and dynamic, any investigation that attempts to evaluate remineralization therapy must simulate the interplay of protective factors and erosive challenges under those dynamic conditions. It must also be able to analyze the effects of the treatment with standardized methodology and measurements. The addition of fluoride in OTC toothpaste has proven to be the gold standard in caries prevention and a great

promoter of enamel remineralization. Several studies have demonstrated that fluoride protects against enamel dissolution during acidic challenges such as frequent energy/sports drinks consumption. Based on the current literature available, it is still questionable whether higher concentrations of fluoride (≥ 5000 ppm) would yield greater protection against dental erosion. Therefore, a different therapeutic agent should be considered to treat the damages from acidic challenges,

CPP-ACP has shown promising results in recent investigations due to its ability to maintain a supersaturated state with calcium and phosphate in the oral environment. *In vitro* studies using SEM, microradiography, and microhardness testing have suggested that CPP-ACP may be able to rebuild apatite crystals in softened enamel and protect against further erosion. However, it remains unclear if CPP-ACP is superior to traditional fluoride remineralization therapy. The formulation of CPP-ACPF attempts to gain the benefits of both therapies by adding 900 ppm fluoride to the existing CPP-ACP paste, and there is some evidence that this may be beneficial.

Currently, there is no formulation or treatment regimen for either fluoride or CPP-ACP that has conclusively demonstrated full protection against enamel erosion in an experimental model that simulates physiologic conditions. Therefore, the purposes of this study were to:

(1) Compare the protective (anti-erosive) effects of 5000 ppm fluoride and amorphous calcium phosphate-casein phosphopeptide as remineralization therapy for enamel subjected to sustained acidic challenges; and

(2) Examine if the combination of both 5000 ppm fluoride and CPP-ACP provides protective effects superior to those of either paste alone.

CHAPTER II: MATERIALS AND METHODS

Ten specimens from National Institute of Science and Technology (NIST) /American Dental Association (ADA) Paffenbarger Research Center's inventory of previously collected and de-identified extracted human teeth, were randomly selected. The collected teeth were sterilized in Streck tissue fixative for two weeks, then rinsed copiously in tap water and transferred to a 0.2% thymol solution 24 hours prior to beginning the study. The teeth were sectioned vertically using a diamond blade (Buehler, Lake Bluff, IL) into 250 μm thickness and sanded with 600 grit silicon carbide abrasive paper (Mager Scientific, Dexter, MI) to create flat 100 μm sections. Measurements of surface erosion, subsurface demineralization, and remineralization can be better visualized on a flat enamel surface rather than on a curved one as seen on natural tooth anatomy.

The sections were embedded in x-ray transparent epoxy to expose one surface to the acidic challenge and remineralization therapy. A copper/rhodium transmission electron microscopy (TEM) mesh grid was positioned on the exposed surface of each specimen parallel to the edge of the enamel and affixed with a small drop of epoxy. Then the specimens were mounted in rectangular molds filled with epoxy. Once cured, each epoxy block was sanded on a polishing wheel (Buehler) with 600 grit silicon carbide abrasive paper to expose the enamel surface of the embedded specimen. Specimen surfaces were then polished with 800, 1200, and 2400 grit silicon carbide paper, keeping each specimen perpendicular to the polishing wheel to avoid beveling the surface. The exposed enamel was thoroughly rubbed with acetone to remove any epoxy that may have contaminated the surface.

The mineral densities of the prepared tooth specimens were captured on VRP-M holographic film (Slavich, Lithuania). The VRP-Mis a fine-grained green-sensitive silver halide emulsion film that has an average grain size of 35-40 nm and resolution power of more than 3000 lines/mm. This was enclosed in a light blocking x-ray holding tray. To capture the image, the tray was placed 30 cm from the Cu radiation source for 30 minutes at 80 kV_p and 3 mA. The exposed film was developed in a JD-2 developer (Integraf, Kirkland, WA) for 120 seconds then rinsed for 30 seconds in deionized water. The processing was stopped in a 1% acetic acid bath for 60 seconds and the film was washed under flowing tap water for 10 minutes. The film was then air dried overnight. The procedures for specimen preparation and microradiograph production are illustrated in Figure 1.

Mineral density of each specimen was measured using the microradiography method developed by Schmuck and Carey (2010), as follows: The developed films were trimmed and fixed to a glass slide for viewing under a Leica MZ16 microscope (Leica Microsystems, Bannockburn, IL) and the digital images were captured with an Evolution MP-5.0 digital microscope camera (Media Cybernetics, Silver Spring, MD). This produces a 12-bit grayscale value for each pixel image, which can be analyzed by the image processing software package (ImageJ, U.S. National Institutes of Health, Bethesda, MD).

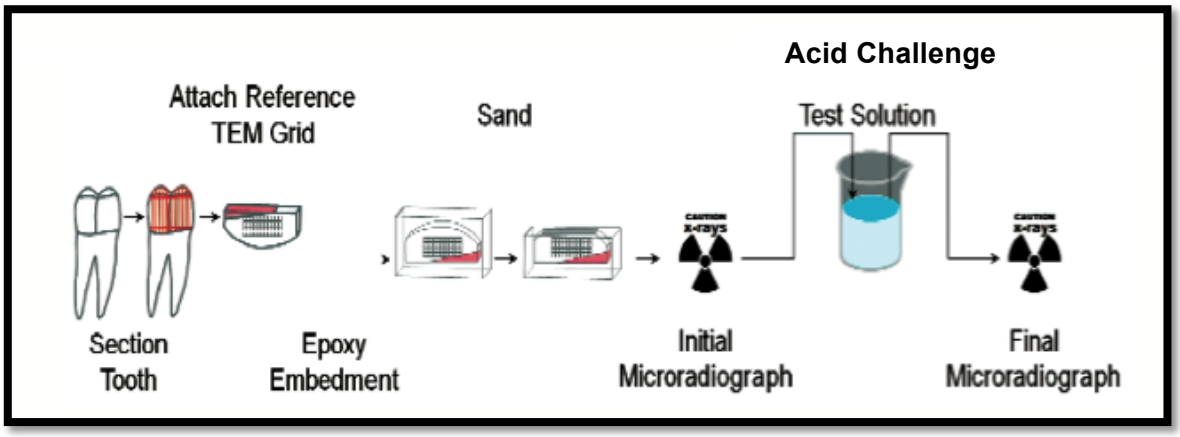

Figure 1. Procedures for specimen preparation and microradiograph production. (Schmuck and Carey 2010; used with permission).

Following the erosive acid challenge formation, the acid was removed with water and mineral density of each specimen was measured as described above. Next, the specimenswere randomly assigned to five groups of 5 specimens for anti-erosion treatment, as outlined in Tables 2 – 4.

Table 1. pH cycling and surface treatment components.

Solution	Composition	pH	Duration
Artificial saliva (Modified Fusayama Saliva) (Mueller 2001)	NaCl (6.8 mmol/L), KCL (5.4 mmol/L), $CaCl_2 \cdot 2H_2O$ (5.4 mmol/L), $NaH_2PO_4 \cdot H_2O$ (5.0 mmol/L), $Na_2S \cdot 9H_2O$ (21 μmol/L), urea (16.7 mmol/L)	7.0	21 hours/day
Erosion solution	1% citric acid solution (4.0 mmol/L)	3.9	30 min/day
Fluoride solution	Various concentrations : (0, 900, 5000) mg/L NaF to create 0, 900, 5000 ppm, respectively	7.0	3-minute applications
CPP-ACP solution	10% amorphous calcium phosphate-casein phosphopeptide solution	7.0	3-minute applications

In order to simulate the oral environment, the specimens were subjected to an *in vitro* pH cycling system between erosive acid challenge and protective factors which mimicked the fluctuation in pH and treatment with oral healthcare products (ten Cate 1982; Carvalho & Cury 1999; Carey, Gove, & Eichmiller 2004).

The erosive challenge consisted of exposing each specimen to an acidic solution (1% citric acid solution at pH 3.9) for 30 minutes each day, followed immediately by application of the protective agents and storage in artificial saliva. This model was used to mimic the oral environment via timed cycling between artificial saliva bathing solution, acidic attack, and various fluoride and CPP-ACP protective rinses. The protective treatments were rendered immediately after the pH cycling acid challenge, according to the schedules listed in Tables 2 – 4. Finally, the specimens were suspended in a buffering solution of artificial saliva (pH 7.0) for storage for the remaining 23 hours in the day (Carvalho & Cury 1999).

The effects of the anti-erosive treatments were evaluated in three phases. In Phase 1, the tooth samples received the anti-erosion treatment application once a week for 14 days (Table 2). In phase 2, the samples received the anti-erosive treatment three times per weeks for 14 days (Table 3). In Phase 3, the samples received the anti-erosive treatment daily for 14 days (Table 4). The pH was recorded and the specimens were evaluated for erosion by x-ray microradiography before each phase of the experiment and after two weeks of treatment to assess the protective potential of the experimental fluoride and CPP-ACP regimens.

Table 2. Phase 1: One anti-erosion treatment application per week (2 week duration).

Treatment Groups	Weekly Anti-Erosion Therapy	Applications
1	No treatment (Control)	Day 1, 8
2	Fluoride 5000 ppm (3 minute) (5000 mg/L NaF)	Day 1, 8
3	CPP-ACP (3 minute) (10% amorphous calcium phosphate-casein phosphopeptide)	Day 1, 8
4	Fluoride 900 ppm + CPP-ACP (3 minute)	Day 1, 8
5	Fluoride 900 ppm (3 minute) (900 mg/L NaF)	Day 1, 8

Table 3. Phase 2: Three anti-erosion treatment applications per week (2 week duration).

Treatment Groups	3 Times Per Week Anti-Erosion Therapy	Applications
1	No treatment (Control)	Days 1, 3, 5, 8, 10, 12
2	Fluoride 5000 ppm (3 minute) (5000 mg/L NaF)	Days 1, 3, 5, 8, 10, 12
3	CPP-ACP (3 minute) (10% amorphous calcium phosphate-casein phosphopeptide)	Days 1, 3, 5, 8, 10, 12
4	Fluoride 900 ppm + CPP-ACP (3 minute)	Days 1, 3, 5, 8, 10, 12
5	Fluoride 900 ppm (3 minute) (900 mg/L NaF)	Days 1, 3, 5, 8, 10, 12

Table 4. Phase 3: Seven anti-erosion treatment applications per week (2 week duration).

Treatment Groups	7 Times Per Week Anti-Erosion Therapy	Applications
1	No treatment (Control)	Daily
2	Fluoride 5000 ppm (3 minute) (5000 mg/L NaF)	Daily
3	CPP-ACP (3 minute) (10% amorphous calcium phosphate-casein phosphopeptide)	Daily
4	Fluoride 900 ppm + CPP-ACP (3 minute)	Daily
5	Fluoride 900 ppm (3 minute) (900 mg/L NaF)	Daily

The enamel mineral density was measured before and after each anti-erosive treatment by contact x-ray microradiography (Schmuck & Carey 2010). Mean (± standard deviation) pre- and post-treatment mineral density (µm/pixel) was calculated for each experimental group. Data were analyzed by two-way ANOVA, and, if significant differences were indicated, Tukey HSD post hoc tests to determine differences in mineral densities (1) within each treatment group from pre- to post-treatment and (2) among the four groups following treatment. Statistical analyses were accomplished using Microsoft Excel 2007 Statistical Analysis ToolPack and verified using Statistical Package for the Social Sciences (SPSS) Version 14 computer software. All statistical significance levels were set at $\alpha = 0.05$.

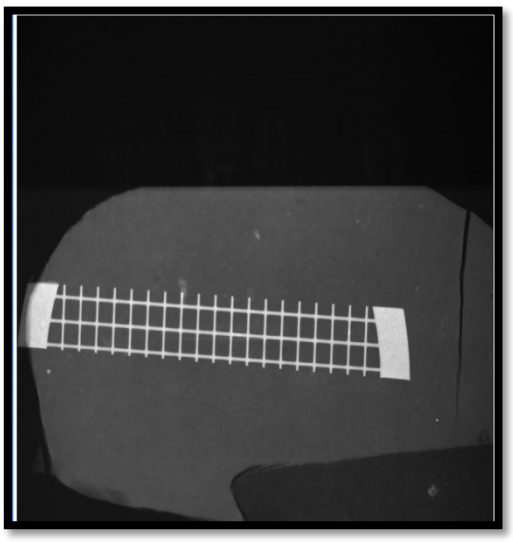

Figure 2- Ante Contact Microradiograph.
This is a sample digital image of the microradiographs of a tooth specimen before acid challenge. The geometric reference grid provided positional orientation for comparison. The images were aligned and vertical profile data were collected.

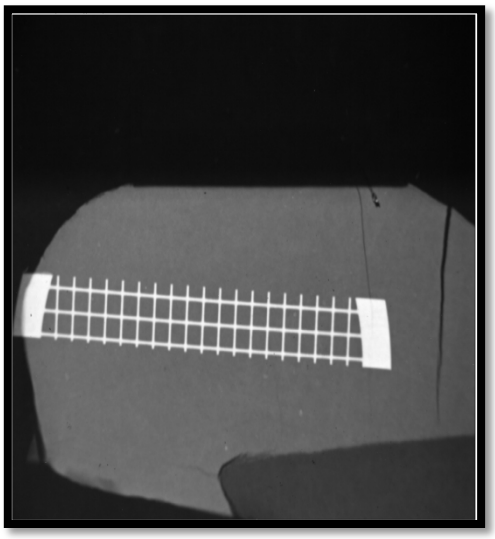

Figure 3. Post Contact Microradiograph
After two weeks experimentation, surface erosion was noted on the exposed enamel after acid exposure and preventive treatment.

The geometric reference grid provided positional orientation for comparison. The images were aligned and vertical profile data were collected. Surface erosion was noted on the exposed enamel surface after acid exposure. These data were plotted within the spreadsheet and offset until the grayscale maximum peaks were aligned. The grayscale values were normalized for each data set by setting the black background to 0 % and the unaffected mineral to 100 % mineral density.

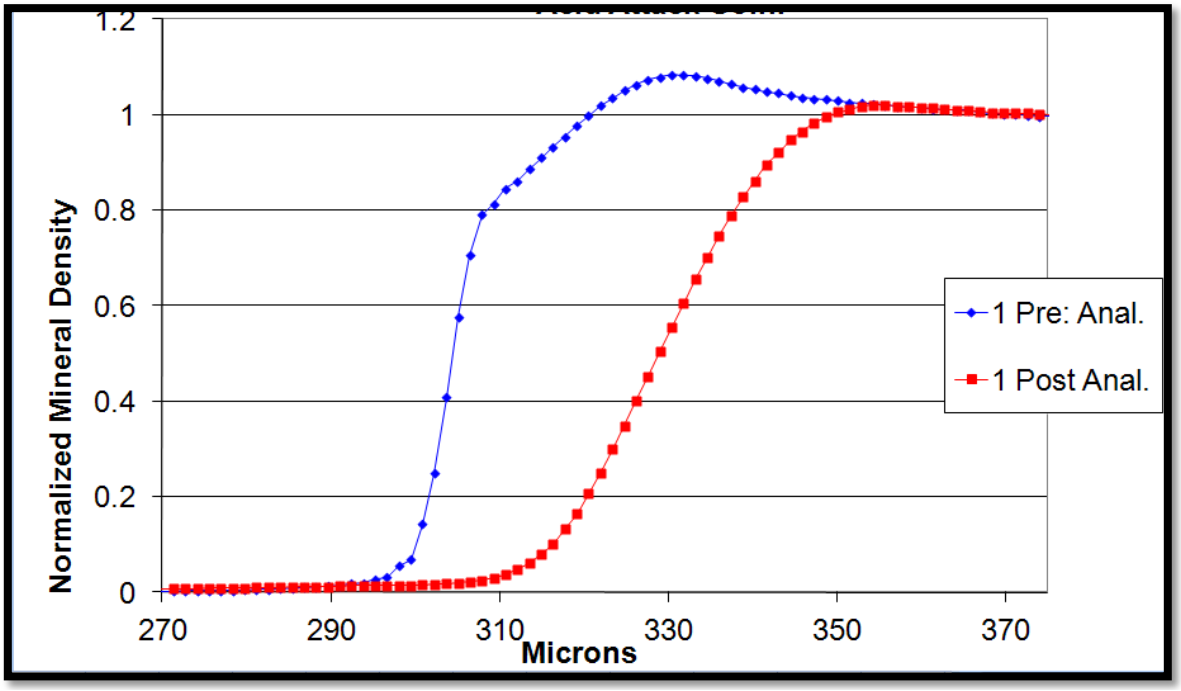

Figure 4. Example of normalization graph of mineral density before and after erosive challenge.

After normalization, the before and after mineral density profiles were plotted on the same graphs. Figure 4 is an example of the normalized mineral density graphs. Radiographs before and after erosion challenge were aligned in Image J Software and plotted as relative mineral density versus distance. The blue plotted values represent in microns the exposed enamel

surface position relative to the edge of the epoxy before the acid challenge. The red line represents the enamel edge position after the acid challenge. Erosion was defined by 20% mineral density (or the 0.2 on the y-axis). In this example, the post analysis of one sample in the control group demonstrated that the acid challenge caused approximately 20 μm of enamel surface loss after one week of pH cycling.

CHAPTER III: RESULTS

Treatment Groups	Phase 1: Weekly Application	Phase 2: Three times a Week	Phase 3: Daily Application
No treatment (Control)	18.81 μm	18.81 μm	18.81 μm
Fluoride 5000 ppm (3 minute) (5000 mg/L NaF)	15.18 ±3.65 μm	8.15 ± 3.65 μm	2.82 ± 5.77 μm
CPP-ACP (3 minute) (10% CPP-ACP)	10.67 ± 3.65 μm	12.65 ± 4.08 μm	9.84 ± 4.08 μm
Fluoride 900 ppm + CPP-ACP (3 minute)	7.99 ± 3.65 μm	31.68 ± 4.08 μm	22.49 ± 3.65 μm
Fluoride 900 ppm (3 minute) (900 mg/L NaF)	22.49± 3.65 μm	5.62 ± 8.16 μm	8.81 ± 4.08 μm

Table 5. Mean (± standard error of the mean) surface erosion (μm).

There was a total of 75 specimens (n=75). The results from this *in vitro* study are presented in Table 5. Two-way analysis of variance showed that the control (no treatment) group demonstrated a mean surface enamel loss of 18.81 (± 14.55) μm. Comparison of erosive loss among the four treatment groups can be seen in Figures 5-7. In Phase 3 testing, daily application of F 5000 ppm demonstrated the least amount of erosion among all phases and treatment groups. F plus CPP-ACP showed more surface loss than control in both Phase 2 and Phase 3.

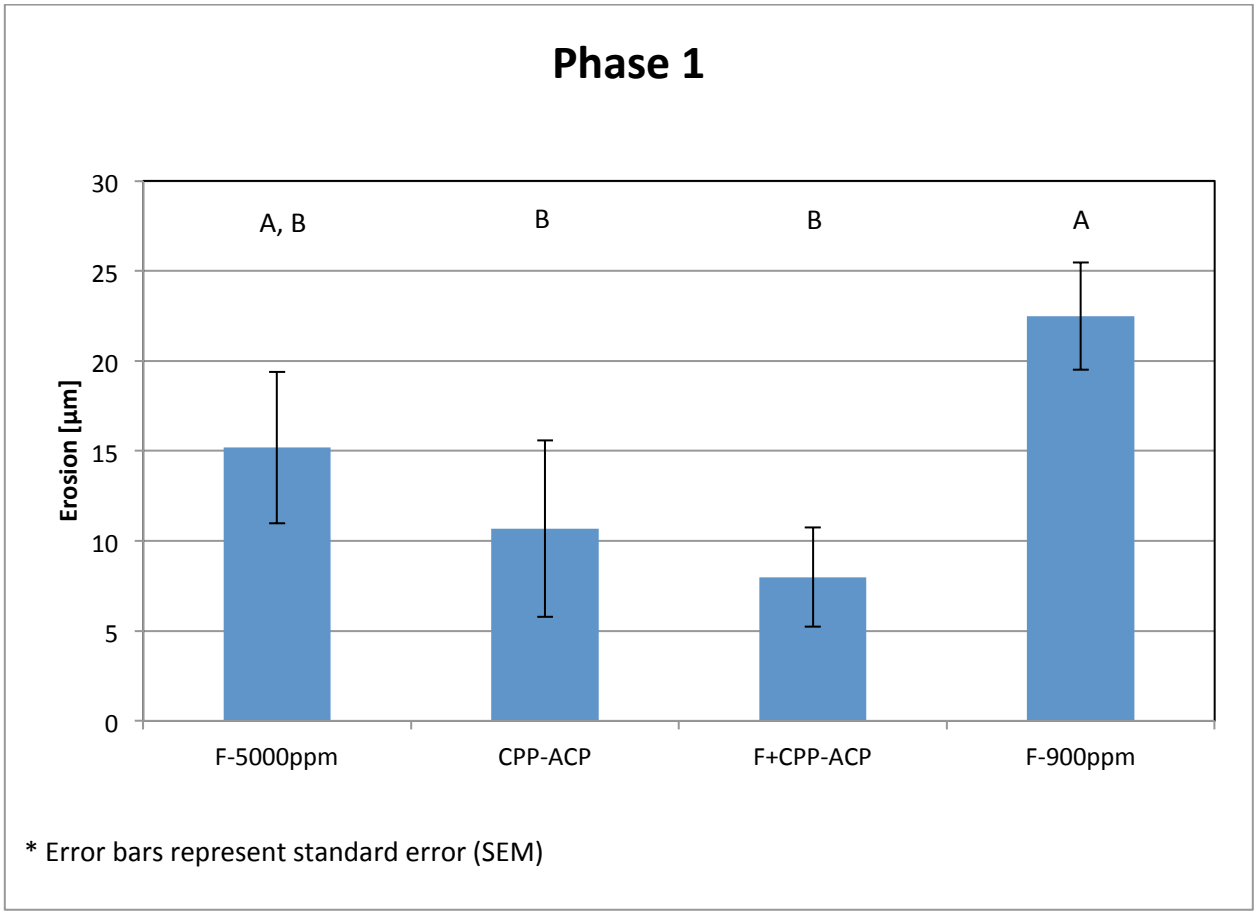

Figure 5. Mean surface erosion after 2 weeks in Phase 1 experimentation. Enamel specimens were exposed to daily acid challenges and received protective therapy once a week. Letters (A, B) denote treatment groups exhibiting statistically non-significant differences in mean surface erosion ($p > 0.05$).

The data were analyzed using a two-way ANOVA, comparing the four different treatment types and various frequencies of application in three phases. In phase 1 (weekly application), a significant difference was noted between CPP-ACP and F +CPP-ACP versus F-900 ppm treatments ($p=0.028$, $p = 0.008$ respectively). F + CPP-ACP demonstrated the best protection against erosion with 7.99 µm surface loss, compared to 22.49 µm when F 900 ppm was applied. CPP-ACP was also more effective than F-900 ppm. Addition of fluoride to CPP-

ACP was not significantly better than CPP-ACP alone. There were no significant differences among the F 5000 ppm, CPP-ACP, and F+CPP-ACP treatments (all $p > 0.17$)

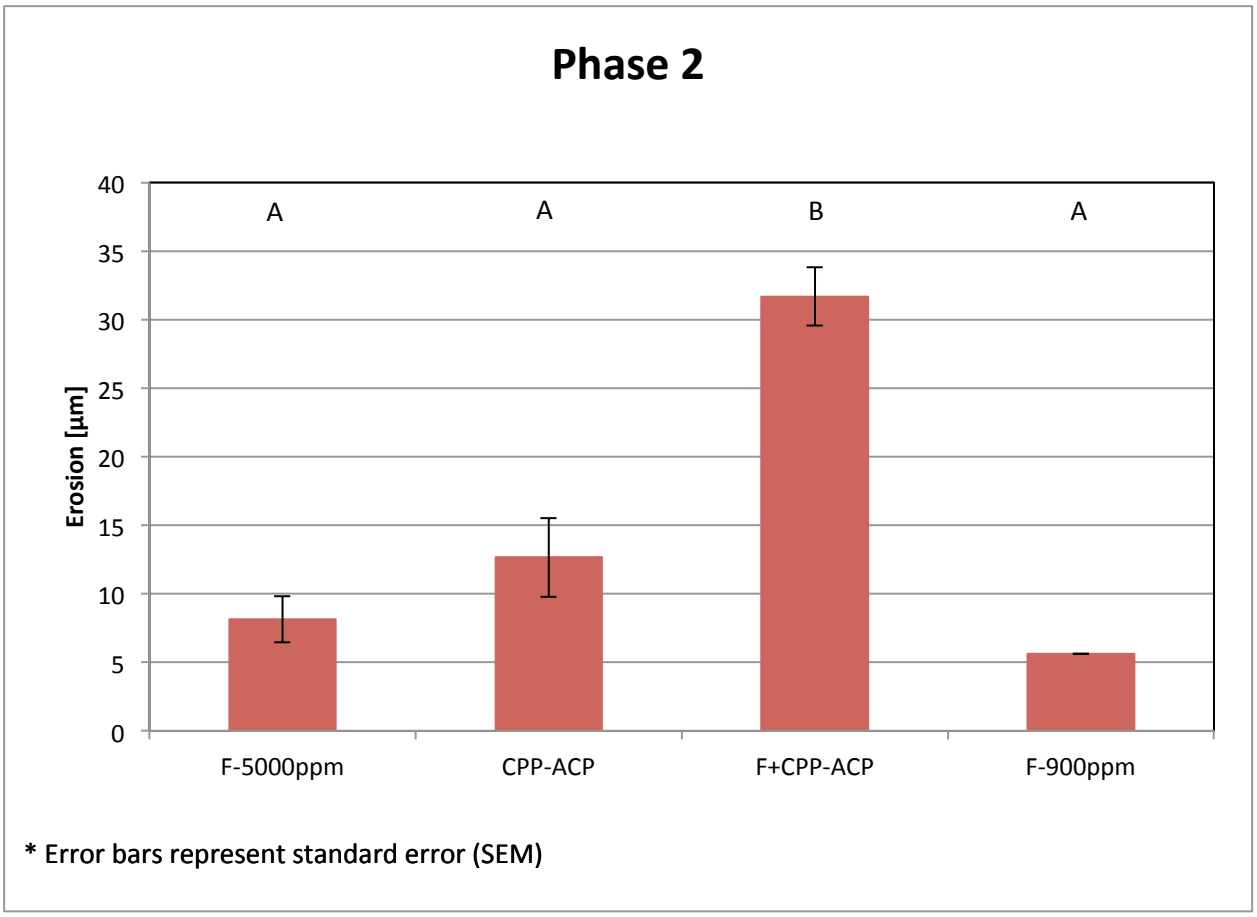

Figure 6. Mean surface erosion after 2 weeks in Phase 2 experimentation. Enamel specimens were exposed to daily acid challenges and received protective therapy three times per week. Letters (A, B) denote treatment groups exhibiting statistically non-significant differences in mean surface erosion ($p > 0.05$).

In phase 2 (application of therapeutic agents three times per week), F + CPP –ACP had significantly more enamel surface loss (31.68 µm) and significantly lower erosion protection compared to the other regimens ($p = 0.007$). However, there were no significant differences in surface loss among the F 5000 ppm, CPP-ACP, and F 900 ppm treatment groups ($p =>0.08$).

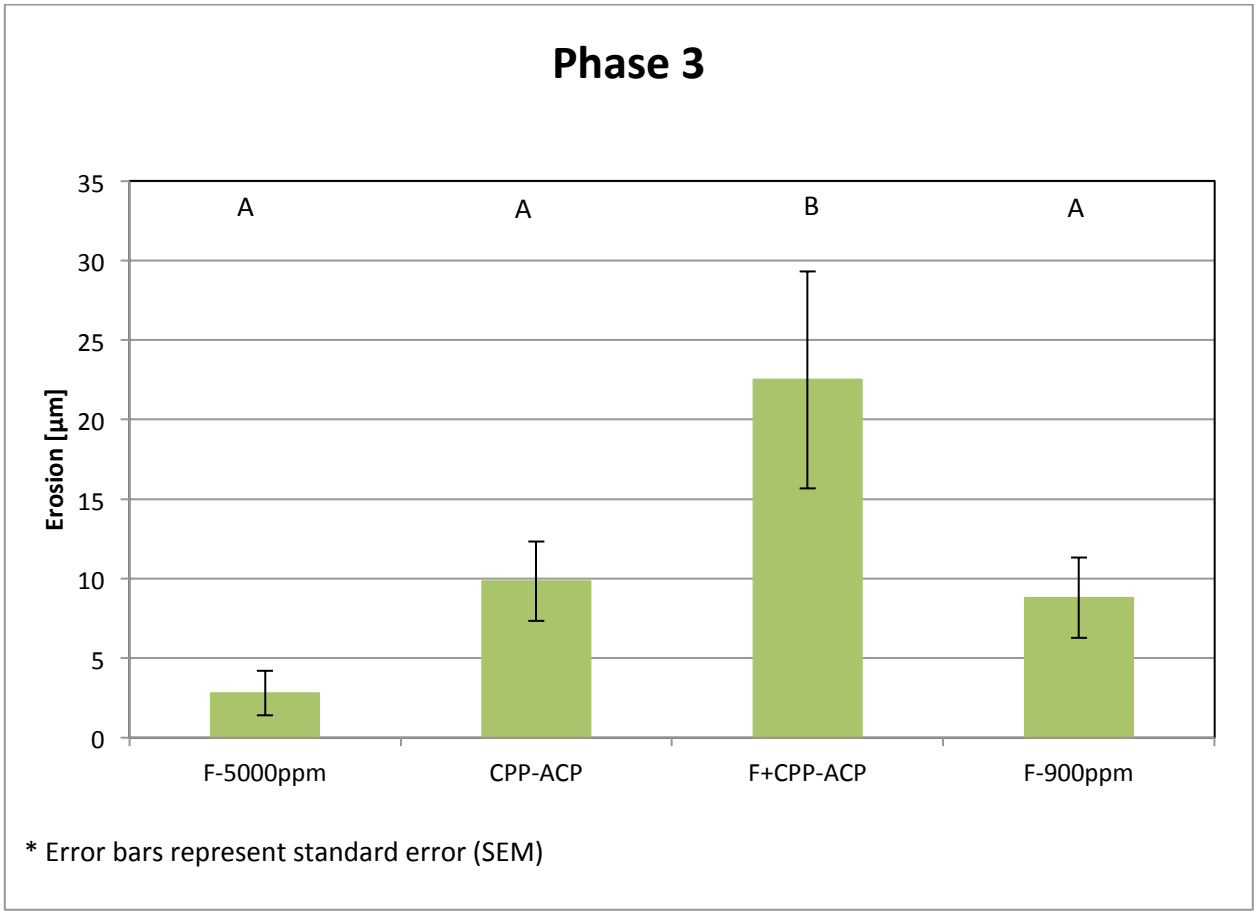

Figure 7. Mean surface erosion after 2 weeks in Phase 3 experimentation. Enamel specimens were exposed to daily acid challenges and received protective therapy daily.
Letters (A, B) denote treatment groups exhibiting statistically non-significant differences in mean surface erosion ($p > 0.05$).

In phase 3 (the protective agents applied daily), F 5000 ppm demonstrated the lowest amount of erosion (2.82 μm) and was significantly better than F+ CPP-ACP, which demonstrated the most surface erosion (22.94 μm) ($p = 0.007$). There were no significant differences noted among the F 5000 ppm, CPP-ACP, and F 900 ppm treatment groups ($p > 0.05$).

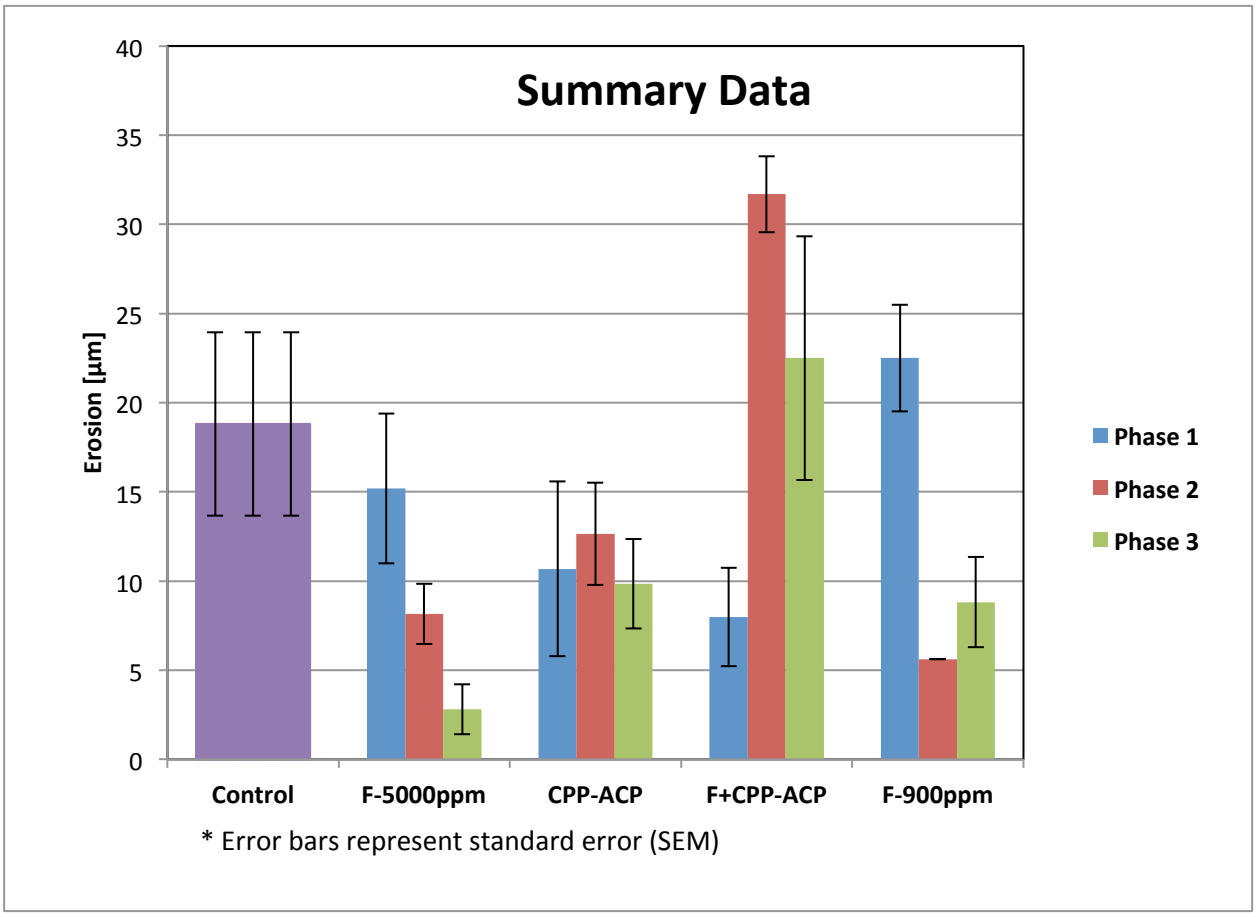

Figure 8. Summary of mean enamel surface erosion for each treatment group and all three phases of treatment.

In general, when enamel specimens were immersed in 1% citric acid (pH 3.9), erosion occurred regardless of therapeutic agent or frequency of application. However, F 5000 and CPP-ACP demonstrated less erosion compared to the control (no treatment) when applied daily, three times per week, or weekly. Daily application demonstrated the most protective effect on enamel surface loss, except in the F + CPP group which had more surface loss than the control.

Comparing the phases and application intervals, daily application of F 5000 ppm appeared to show the least surface erosion. However, it was not significantly better than three times per week or weekly application. CPP-ACP and F 900 ppm also showed no significant differences when applied daily, threes per week, or weekly. On the other hand, F900 + CPP-ACP did result in less erosion when applied only once a week and was significantly better than more frequent applications

Despite the limited sample size per treatment group (n=5), significant differences were noted among the three different phases of treatment ($p=0.05$). Phase 3 with daily application of protective demonstrated the least amount of surface erosion and was significantly better than weekly application, but was not more effective than three times a week.

CHAPTER IV: DISCUSSION

Dental erosion is the acid dissolution of mineral, which causes surface tooth loss. The acid can be from intrinsic systemic sources or extrinsic consumption of food or beverages. Clinically, early erosive lesions may start as smooth, shiny, and mildly concave defects on the occlusal surfaces; then progress to a dull surface that lacks luster without extrinsic staining. If left untreated, tooth morphology is lost as more surface tooth structure is lost. Patients may present with exposed dentin and complain of generalized cold sensitivity.

The chemical mechanism of erosion can occur either by hydrogen ion dissociation from the acid or by binding the calcium from the tooth surface by the anion from a chelating agent. Citric acid was chosen for this investigation's acidic component because it is also one of the main ingredients used in acidic beverages such as energy drinks, soft drinks and sports drinks. The pH of the citric acid used in this experiment was 3.9, which is lower than enamel's critical pH of 4.5 at which erosive demineralization can occur. Citric acid is a very potent erosive agent because it acts as both a dissociated hydrogen ion and chelating acid that has the ability to complex calcium ions from enamel apatite and draws it out of the surface causing erosion (Lussi 2006). It has been shown to have more destructive erosive capacity than hydrochloric and phosphoric acid due to its calcium binding chelating ability (West 2000; Wiegand, Stock, Attin, Werner 2007). Hence, surface erosion can occur in the enamel specimens after the daily 30-minute citric acid challenge.

Schmuck and Carey (2010) noted that a single four-hour acid wash of similar citric acid concentration created pure erosion without demineralization. In addition, the authors demonstrated that the microradiography and ImageJ software were capable of accurately

measuring mineral density loss when there were pure erosive lesions. Demineralization would require a different and more complex form of measurement that was not utilized in this investigation. In the current study, the enamel specimens sustained a total exposure time of 7 hours over 14 days. Since the investigation attempted to simulate oral conditions, it would be unrealistic to maintain acid contact on the enamel specimens for four consecutive hours. However, as a result of the intermittent acid exposure, the data collected showed some demineralization along with the erosive lesions that could have affected the mineral density calculations. The objective of this study was to compare the protective effects against surface erosion; therefore was no attempt to calculate or compare the amount of subsurface demineralization that developed in the samples.

Despite the limited sample size per treatment group (n = 5), when comparing phases only (frequency of protective agent application) significant differences were noted among the three different phases of treatment ($p = 0.05$). Phase 3 with daily application of protective agents demonstrated the least amount of surface erosion and was significantly better than weekly application, but was not more effective than three times a week. This is somewhat contradictory to Ganns and colleagues' (2001) finding that increased frequency of fluoride rinse was more effective in remineralizing lesions.

Given fluoride's reliable history as a remineralization enhancer, it was not surprising that the results echoed the findings by other studies (Mukai and colleagues 2001; Baysan and colleagues 2001; Garcia and colleagues 2010) which demonstrated high concentration fluoride's potential to reduce erosion by decreasing surface demineralization. The resultant effect may have formed a more acid-resistant fluorapatite layer that protects it from further

acid challenges. Evidently, the least amount of erosion was noted in the group that received daily application of F 5000 ppm and was significantly better than the control. F5000 was also more effective than CPP-ACP when applied more three times or daily, but demonstrated no difference when applied only once a week. Pulido and colleagues (2008) also found that F 5000 was more protective than CPP-ACP against demineralization of enamel sections.

The CPP-ACP test group also demonstrated less erosion than the control group. However, it was not significantly better based on the limited number of samples, which was a notable limitation in this investigation. Similarly, Panich and colleague (2009) were able to demonstrate that 3 minute application of CPP-ACP immediately after acid challenge was significantly better than the control.

Another objective of this study was to investigate the effectiveness of combining two promising protective agents, fluoride and CPP-ACP. The data failed to indicate improved protection when the treatment combined F-900ppm and CPP-ACP. In fact, when F+CPP-ACP was applied more frequently, it demonstrated more surface loss than the control ($p<0.08$). Either F-900 ppm or CPP-ACP was more effective when used alone. This is contrary to the results from Reynolds and colleagues (2008), which demonstrated significantly higher remineralization when CPP-ACP was added to F 1100 ppm dentifrice compared to either fluoride dentifrice alone.

For future attempts to repeat a similar method design, it should be mentioned that specimen preparation required much diligence and patience due to the sectioning and handling of thin 100 μm enamel sections. To appreciate the thickness of 100 μm, one can compare it to the thickness of a sheet of paper, which is 90 μm, or a human hair, which on average is also 100

μm. Initially, each 250 μm section from the tooth was carefully sanded on both sides in order to create uniform flat plane 100 μm sections. This technique-sensitive process of tooth sectioning, sanding, and epoxy preparation resulted in many unusable enamel sections. Brittle enamel fracture was the main cause. The thin sections were difficult to manipulate and resulted in enamel fracture at the dentinoenamel junction (DEJ) during handling, while removing it from the sanding piston, and during positioning on the epoxy slides. Moreover, squeezing forceps to handle the sections may damage the delicate 100 μm sections. Hence, a small vacuum suction tip was used instead, to transfer the sections when they were placed under high magnification to be cut into smaller pieces and to the epoxy blanks for fabrication. The TEM grids were also transferred in a similar manner.

Occasionally samples also contained multiple craze lines within the enamel, rendering them unsuitable for experimentation. Enamel is inherently brittle when it is thin and unsupported by dentin. Dehydration of the enamel sections may have also played a role in the fractures. In order to minimize dehydration, extracted teeth and prepared sections were stored in distilled water. The overall, attrition rate of the samples prepped was nearly 50%.

During evaluation of the protective effects of the test agents, the amount of erosion or enamel loss was calculated by using (ante) baseline enamel profile level minus the enamel profile level after (post) two weeks of experimentation. Since the original enamel was sanded flush with the epoxy edge, any erosion that occurred would be positioned below the epoxy edge, and the calculations resulted in a positive value for the amount of enamel lost in μm. After calculation of all collected raw data (not included), some negative values (9 out of 75 total sample preps) were noted which would represent a “surface gain.” Since it is impossible to

"gain" enamel from the proposed treatments, those negative values were considered outliers and were not used in the calculation of means. Despite the apparent visual enamel loss in the microradiographs, the erosion value remained negative after repeated plotting of the grayscale values. Possible explanations for this phenomenon cannot be determined at this time, and deserve further investigation.

With prolonged acid exposure, a visible surface loss can be seen and the mechanical properties of the remaining tooth structure are also affected. The surface has less microhardness and softens as result of mineral loss. Acid challenges cause mineral dissolution that extends a few micrometers below the surface, which has been referred to as softening. This softened enamel surface is more likely to sustain mechanical abrasion from brushing and occlusion (Lussi 2006; Magalhaes & colleagues 2009). However, if the acid is neutralized and the softened enamel is exposed to a protective remineralization agent or to buffering saliva for adequate amount of time, it can remineralize and regain mechanical strength (Lussi 2006; Oshiro 2007; Magalhaes 2009).

In addition to chemical dissolution resulting from acid and chelating agents, behavioral and biological factors play an integral role in dental erosion. One limitation of this investigation was that it did not fully account for factors such as aggressive oral hygiene practices, salivary flow rate or buffering capacity, acquired pellicle, and dental anatomy. The acquired pellicle is a bacteria-free biofilm composed of mucin, glycoprotein and proteins (Martin 2009). Artificial saliva storage was the chose storage medium to simulate the remineralization and buffering capacity of the intraoral environment after the 30-minute 1%

citric acid challenge. However, it cannot duplicate natural saliva's ability to form a pellicle, which has enamel protective functions.

A second limitation of the study design was the lack of pellicle layer on the enamel surface. In vivo, the acid must penetrate the acquired pellicle before it can erode surface enamel and dentin. The pellicle restricts acid diffusion and ion dissolution from the tooth surface and may act to diminish the effects of dietary acids (Lussi 2006; Oshiro 2007; Panich 2009). Therefore, the rate and amount of erosion in this investigation may be exaggerated compared to the oral environment. The presence of a pellicle layer would act as a protective barrier against acid diffusion to the tooth surface and slow the rate of erosion. It is also possible that the lack of pellicle may have affected CPP-ACP from adhering, since it usually binds to the tooth surface via the pellicle and bacteria layer (Reynolds 2009).

Another notable limitation in this investigation is the method of the acid challenge. The 30-minute acid exposure time utilized may not be realistic due to the nature of the constant immersion. During human consumption of acidic beverages, the liquid is not held in the oral environment for that duration of time. However, soda swishers may hold the beverage in the mouth for several minutes before swallowing and this process may repeat for over an hour or as long as it takes the to finish the beverage. It is conceivable that the sipping habit can potentially subject the enamel surface to acidic challenge for 30 minutes or greater with intermittent saliva buffering. This investigation also did not account for such periodic saliva lavage and buffering.

Furthermore, inconsistencies found in the results may be due to the individuality of native enamel in tooth specimens. Some tooth specimens may contain variations such as

demineralized areas, enamel defects, and acquired fluorapatite veneer that may affect the rate of erosion during the acid challenge. The enamel specimens used in this study were sectioned from non-carious premolars and molars. Care was taken to select teeth with minimal or no decalcification spots. In order to minimize the variations inherent in the collected teeth, the enamel specimens were sanded and polished to remove native enamel and expose the underlying crystals to standardize the tested surface. During polishing, special care was taken to ensure the exposed enamel surfaces were perpendicular to the sanding wheel to avoid bevels at the edge. Beveled enamel may give false mineral density gradient readings.

CHAPTER V: CONCLUSIONS

With the increasing trend of sports drink and soda consumption, acid-induced dental erosion is becoming a greater dental health concern. Both primary and permanent dentitions can be affected due to widespread consumption and lifestyle habits associated with prolonged exposure to the acids found in these beverages. Since erosion leads to irreversible loss of surface enamel, it poses a challenging scenario for dental professionals. Often the condition is not diagnosed until there are moderate to severe lesions (Lussi 2006). Mild lesions are difficult to recognize in the early stages and often regarded as within normal limits. If the lesions are allowed to progress with no attempts to educate patients and modify their destructive behaviors, the consequences are detrimental. Severe dental erosion poses irreversible and generalized hard tissue deterioration, requiring complex treatment planning and compliance to be successful.

Based on the results from our pilot study, none of the protective agents was able to completely prevent surface erosion from occurring when enamel specimens were challenged with 30-minutes of daily immersion in 1% citric acid. However, daily application of F 5000 ppm and CPP-ACP therapy was more protective than three times per week and weekly applications.

Within the limitations of the methodology of this study, we can suggest that daily application of fluoride 5000 ppm demonstrated the most promising protection against acid erosion. Fluoride provides protection by replacing the leached on $Ca^{2+}$and PO_4^{3-} ions with F- to form a stronger, more acid resistant fluorapatite crystal than the original hydroxyapatite. As an additional benefit, fluoride can provide relief for dentin hypersensitivity caused by thinning enamel or exposed dentin.

Although it was not more protective than F-5000 ppm, CPP-ACP also demonstrated potential to reduce erosion. Clinicians should consider prescribing one or the other as a protective agent for daily application. However, it is not advisable to prescribe both at the same time since the combination was least effective.

Due to the limited sample size of this *in vitro* pilot study, it is recommended that future investigations should include larger sample sizes, varied therapeutic regimens and intervals, and extended (beyond two weeks) acid challenge periods. In addition, utilizing both stronger and weaker acids may aid to more precisely characterize the potential protective effects of fluoride and CPP-ACP agents. Other modes of applications should be considered, such as varnish or addition of CPP-ACP to sports drinks.

While high concentration fluoride may provide some protection against acid induced dental erosion, the preliminary results still show progressive surface loss with only 30 minutes of acid exposure per day. Therefore, it is crucial for dental professionals to recognize the initial signs and symptoms of dental erosion. Early warning signs should not be ignored, dental professionals need to have a discriminative eye and interview high risk patients regarding their dietary acid consumptions, contributing systemic diseases and habits to identify destructive factors (Magalhaes & colleagues 2009; Lussi 2006). Ultimately, since the etiology is multifactorial, combining adequate buffering to neutralize the acidic environment, daily application of high concentration fluoride therapeutic agent and patient education for behavior modification may be the best preventive measures.

APPENDIX A

These are photographs of severely eroded occlusal surfaces with classic concave lesions. The patient in the above photograph is an admitted soda swisher. She holds the soda in her mouth until the bubbles subsides before she swallows it.

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